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# Chloroplast DNA Polymorphism of the Medicinal Plant *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. in the Southern Part of the Russian Far East

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**Abstract**—The polymorphism of the nucleotide sequences of the *trnL–trnF*, *rpl16 intron*, and *matK* regions of chloroplast DNA of the medicinal plant *Eleutherococcus senticosus* in the southern part of the Russian Far East (RFE) has been studied. The level of genetic diversity was found to be high in the populations of Primorskii krai and average on Sakhalin Island. Hierarchical analysis of the molecular variance showed very low and insignificant genetic differentiation between the populations of these two regions ($\Phi_{CT} = 0.043$, $P > 0.479$); more than 62% of all genetic variability accounted for the intrapopulation component ($\Phi_{ST} = 0.377$, $P = 0.000$). Eleven haplotypes were identified in the RFE, one of which was unique and two of which were rare for the species. All haplotypes known for *E. senticosus* form a single group with minimal divergence between each other.

**Keywords:** *Eleutherococcus senticosus*, Siberian ginseng, genetic diversity, chloroplast DNA, genealogical relationships

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## INTRODUCTION

*Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. [=*Acanthopanax senticosus* (Rupr. et Maxim.) Harms] or Siberian ginseng is a deciduous, sparsely branched thorny shrub that is found only in northeast Asia: in the Russian Far East (RFE), in northern and northeastern China, the northern and central parts of the Korean Peninsula, and the Japanese island of Hokkaido (Zhuravlev and Kolyada, 1996). Of all the representatives of the family Araliaceae in the Russian Federation, *E. senticosus* has the northernmost distribution, occupying territories in Primorskii krai, central and southern Khabarovsk krai, southeastern Amur oblast, and south of the Schmidt Line on Sakhalin Island. It grows in groups and thickets of varying density in the undergrowth of coniferous and mixed forests on mountain slopes and river valleys. *Eleutherococcus senticosus* is a valuable medicinal plant and is included in the State Pharmacopoeia of the Russian Federation (*Gosudarstvennaya…,* 2018). Its aboveground and underground parts contain glycosides, the main active ingredients, as well as carbohydrates, polysaccharides, phenolic compounds, trace elements, etc. Preparations based on it are widely used in adaptogenic, tonic, restorative, stimulating, antioxidant, antimetastatic, and other means in the health of humans and farm animals (Syutkina et al., 1992; Zhuravlev and Kolyada, 1996; Kuznetsov and Gorshkov, 2016; Ptazhynska and Załuski, 2020). The rhizome and roots of *E. senticosus* are most often used as raw materials.

Uncontrolled procurement of *E. senticosus,* especially near populated areas, industrial logging, and forest fires, leading to changes in the ecological regime and degradation of forest vegetation, contribute to the depletion of *Eleutherococcus* resources. In Korea, *E. senticosus* is already listed as an endangered plant, and in China it is listed as a vulnerable species in the Red Book (Zhou et al., 2004; *Korean…,* 2014). Molecular genetic studies of natural *Eleutherococcus* populations in these regions indicate a decrease in the genetic diversity of the species. Thus, an analysis of isoenzymes in *E. senticosus* showed that populations in China (Zhou et al., 2004) were characterized by low polymorphism ($P = 26.9\%$) and an average level of interpopulation differentiation ($G_{ST} = 0.383$), and populations in Korea (Huh, M.K. and Huh, H.W., 2005) had a medium level of polymorphism ($P = 47.8\%$). In both regions, *E. senticosus* showed a deficiency of heterozygotes. Comparative analysis of nucleotide polymorphism of the ITS region of the ribosomal operon of nuclear DNA revealed that the

**Table 1.** Studied populations of *Eleutherococcus senticosus* and parameters of genetic diversity according to cpDNA data

| Sample code | Location (number of samples) | Haplotype (number of samples) | Diversity (standard deviation) | |
|---|---|---|---|---|
| | | | haplotypic *h* | nucleotide π |
| Primorskii krai | | | | |
| ACD | Vladivostok area, 11 km (11) | Esc1 (1), Esc2 (1), Esc3 (3), Esc4 (1), Esc5 (3), Esc6 (2) | 0.873 (0.071) | 0.0011 (0.0007) |
| OCN | Ocean Ridge, Vladivostok area (15) | Esc3 (9), Esc4 (3), Esc5 (1), Esc6 (1), Esc7 (1) | 0.629 (0.125) | 0.0010 (0.0007) |
| KP | Kedrovaya Pad Nature Reserve (7) | Esc6 (7) | 0.000 (0.000) | 0.0000 (0.0000) |
| RTT | Rettihovka settlement area (6) | Esc9 (4), Esc11 (2) | 0.533 (0.172) | 0.0024 (0.0015) |
| VTZ | Gamova Peninsula, environs of the village of Vityaz (2) | Esc8 (1), Esc10 (1) | – | – |
| KMN | Vicinity of the village of Kamenushka, right bank of the Volkhushka River (2) | Esc4 (1), Esc5 (1) | – | – |
| Total sample (43) | | Esc1–Esc11 | 0.847 (0.031) | 0.0015 (0.0008) |
| Sakhalin Island | | | | |
| YSK | Environs of Yuzhno-Sakhalinsk (13) | Esc5 (1), Esc6 (1), Esc7 (9), Esc8 (2) | 0.526 (0.153) | 0.0004 (0.0005) |
| NVL | Nevelsk area (2) | Esc6 (1), Esc9 (1) | – | – |
| Total sample (15) | | Esc5–Esc9 | 0.638 (0.129) | 0.0025 (0.0014) |

sequences of *E. senticosus* from Korea and China were identical and had 99.2% homology with the sequence of *Eleutherococcus* from Russia (Han et al., 2006).

However, analysis of the variation of three chloroplast DNA (cpDNA) regions, *trnL–trnF, rpl16,* and *matK,* revealed high levels of genetic variation in *E. senticosus* in northern and northeastern China (Wang et al., 2016). The authors of the study suggested that the increase in local genetic diversity in *E. senticosus* populations in northeastern China could have resulted from the mixing of genetic lineages from multiple refugia of the Last Glacial Maximum (LGM).

The aim of this work is to study the genetic variability of *E. senticosus* populations in the Russian Far East using data on the nucleotide sequences polymorphism of the *trnL–trnF,* the *rpl16* intron, and the *matK* gene of cpDNA, and to conduct an analysis of intraspecific differentiation using data from GenBank/NCBI.

## MATERIALS AND METHODS

We analyzed 58 plants from eight natural populations of *E. senticosus* in the southern part of Primorskii krai and Sakhalin Island (Table 1). Given the shrub's ability to reproduce by root suckers, plants located more than 70 m apart from each other were selected from each population. Individual preparations of total DNA were isolated from leaves using the method of (Isabel et al., 1993) with minor modifications.

The regions *trnL–trnF, rpl16,* and *matK* were amplified with primers c and f (Taberlet et al., 1991), F71 and R1516 (Small et al., 1998), and those indicated in the work by Zuo (Zuo et al., 2011). For all regions, the same polymerase chain reaction (PCR) program described by Shaw (Shaw et al., 2007) was used as the most efficient. The PCR conditions were as follows: primary DNA denaturation at 80°C for 5 min, then 30 cycles, including three stages (denaturation at 95°C for 1 min, primer annealing at 50°C for 1 min, then increasing the temperature at a rate of 0.3°C/s to 65°C and synthesis at 65°C for 4 min); final synthesis for 5 min at 65°C. The nucleotide sequences of the forward and reverse strands were determined on an ABI 3500 genetic analyzer (Applied Biosystems, United States), then edited in Chromas 2.4.4 (http://technelysium.com.au/wp/chromas), and aligned and combined into one matrix using the MEGA ver. X software package (Kumar et al., 2018). The number of haplotypes, nucleotide (π) and haplotype (*h*) diversity (for populations with five or more samples), distribution of genetic variability within and between populations/population groups (AMOVA), and pairwise genetic distances ($F_{ST}$) were calculated using the Arlequin v. 3.5 program (Excoffier and Lischer, 2010). The haplotype sequences of each region were deposited in ENA/EMBL-EBI under accession numbers LR897820–LR897830, LR897845–LR897855, and LR897868–LR897878. Reconstruction of the genealogical relationships of *E. senticosus* haplotypes from RFE and China was analyzed using the median-joining (MJ) method in the Network 10.1 program (Bandelt et al., 1999). Sequences *trnL–trnF, rpl16,* and *matK* of *Eleutherococcus* growing in China (Wang et al., 2016) were retrieved from GenBank/NCBI (haplotypes H1–H15, accession numbers KU378054–KU378101). Sequences of all samples from each region were then aligned, trimmed, and combined into a common matrix for analysis. Each deletion/insertion, regardless of its size, was coded as a single mutational event.

## RESULTS AND DISCUSSION

The *trnL–trnF* region in the studied RFE samples is represented by three length variants, 935, 937, and 944 bp, due to the presence of two indels (2 and 9 bp), and one C↔T substitution was found at position 836 bp. Intron *rpl16* contained four substitutions and a 6 bp indel; the size of the sequences was 887 and 893 bp. The length of the *matK* sequences in all samples was 850 bp. Six substitutions were found in this region. The total length of the combined sequences of the three cpDNA regions was 2689 bp, including 2661 monomorphic and eleven variable nucleotide substitutions, of which eight were informative (maximum parsimony method), and three indels. The combination of identified mutations yielded eleven haplotypes (Esc1–Esc11), all of which are found in populations of Primorskii krai, but only five (Esc5–Esc9) on Sakhalin Island (Table 1). In Primorskii krai, the ACD and OCN samples from the vicinity of Vladivostok were the most variable, in which six and five haplotypes were identified, respectively, and the KP population from the Kedrovaya Pad Nature Reserve was monomorphic. In the YSK sample from the vicinity of Yuzhno-Sakhalinsk, four haplotypes were identified, with medium haplotype and low nucleotide diversity. For the overall sample from Primorskii krai (43 samples), the haplotype diversity was higher and the nucleotide diversity was lower than for the overall sample from Sakhalin Island (15 samples) (Table 1). The observed level of genetic diversity in the populations of *E. senticosus* of the RFE is comparable to that in populations of Northern and Northeastern China ($\pi$ from 0.00044 to 0.00154, $h$ from 0.389 to 0.889) (Wang et al., 2016).

Analysis of molecular variance (AMOVA) showed that in *E. senticosus* of the RFE, more than 80% of all genetic variability is accounted for by the intrapopulation component ($\Phi_{ST} = 0.194$, $P = 0.000$). Hierarchical analysis of molecular variance showed low and statistically insignificant genetic differentiation between the populations of Primorskii krai and Sakhalin Island ($\Phi_{CT} = 0.043$, $P > 0.479$). Within each geographic region, between-population variability accounted for approximately 35% ($\Phi_{SC} = 0.349$, $P = 0.000$), and within-population variability accounted for more than 62% ($\Phi_{ST} = 0.377$, $P = 0.000$). Low population differentiation and weak genetic structuring of populations were determined for *E. senticosus* in China ($G_{ST} = 0.278$, $F_{CT} = 0.261$) (Wang et al., 2016).

To reconstruct the genealogical relationships of *E. senticosus* haplotypes from the RFE and China, a common matrix was created, the length of which after alignment was 2345 bp. Haplotypes Esc1, Esc3, Esc5, Esc7, Esc8, and Esc 10 were identical to haplotypes H3, H12, H9, H2, H1, and H8 of the Chinese samples, respectively, and Esc6 was identical to three haplotypes—H4, H6, and H7. A total of 17 haplotypes was found, of which seven (Esc1, Esc3, Esc5–Esc8, Esc10) were common to *E. senticosus* of the RFE and China, while six (H5, H10–H11, H13–H15) were found only in Chinese samples, and four (Esc2, Esc4, Esc9, Esc11) were only in the RFE samples.

The distribution of haplotypes in 28 populations of *E. senticosus* of the RFE and China (Wang et al., 2016) is shown in Fig. 1a. Haplotypes Esc6, Esc8, and H5 are widespread across the species range (in 17 (61%), 16 (57%), and 13 (46%) populations, respectively). For the RFE, haplotype Esc8 was rare. In Primorskii krai, the greatest number of plants were found with haplotypes Esc3 and Esc6, while Esc7 was unique. On Sakhalin Island, haplotype Esc7 dominates and is rare in three populations (WYL, CH, and WQ) of northeastern China (Wang et al., 2016). It is highly likely that this haplotype is more characteristic of northern latitudes. It should be noted that the RFE revealed one unique haplotype (Esc2) in the ACD population and two rare ones for the species: haplotype Esc10 was found once in the VTZ population on the Gamov Peninsula (Primorskii krai) and in the Chinese BS population in the Changbai Mountains, and Esc11 was found only in two samples in the RTT population of Primorskii krai.

In the median network of genealogical relationships (Fig. 1b), all haplotypes of *E. senticosus* form a single group with minimal divergence between them (from one to three mutational steps) and with two star-shaped structures with haplotypes Esc6 and H15 in the center. The phylogeographic structure of the species is not expressed. Nevertheless, an analysis of paired $F_{ST}$ revealed weak but statistically significant genetic differentiation between geographic groups of populations. The greatest differentiation was observed between populations of Sakhalin Island and Northern China ($F_{ST} = 0.276$). Approximately equal values were found for the pairs Primorskii krai–Sakhalin Island, Primorskii krai–Northern China, and Sakhalin Island–Northeastern China ($F_{ST}$ was 0.194, 0.166, and

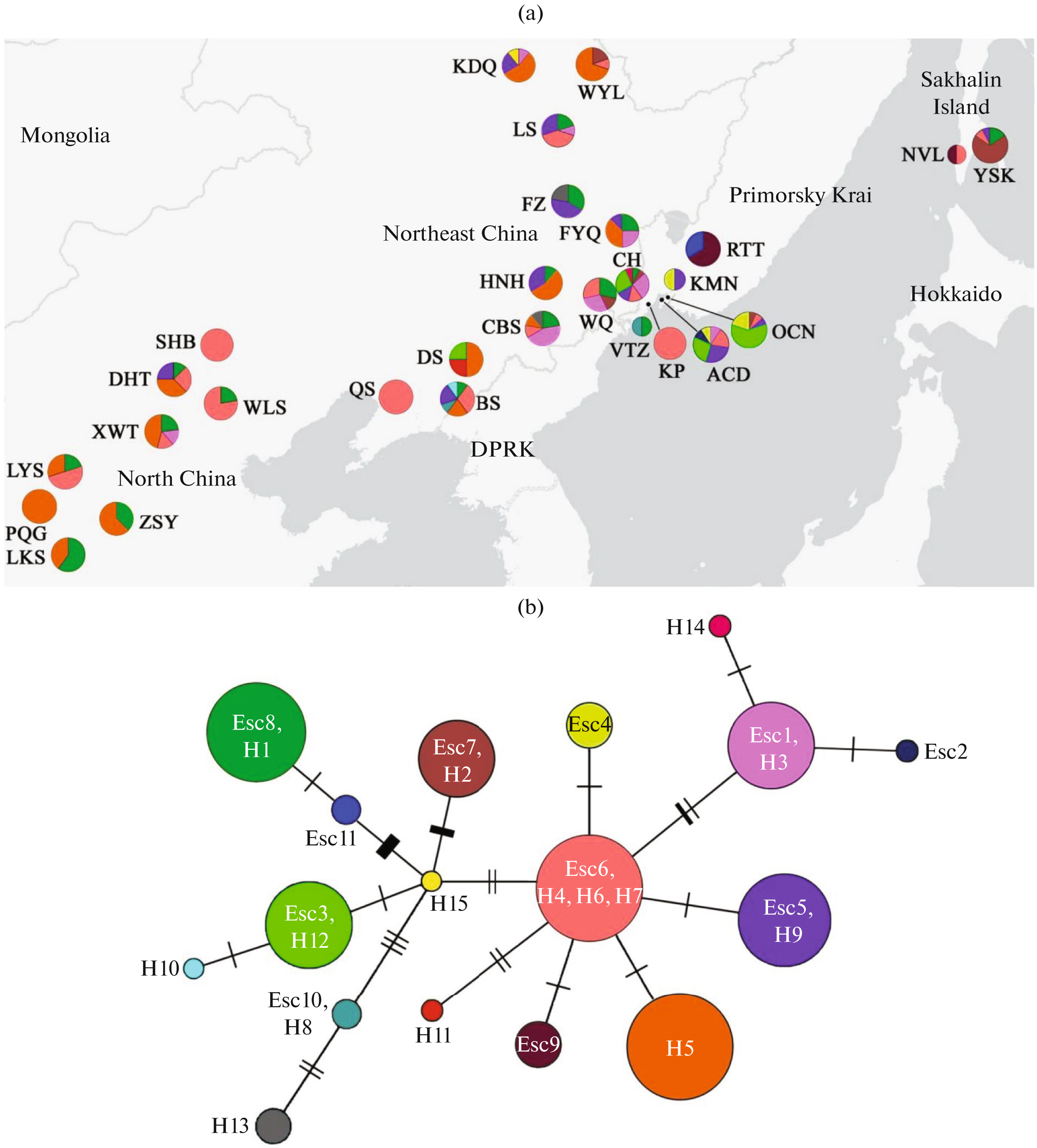

**Fig. 1.** (a) Distribution of cpDNA haplotype frequencies in *E. senticosus* RFE and Chinese populations. The number of samples and the code and location of populations in China are taken from the work (Wang et al., 2016). (b) A genealogical network of haplotypes constructed using the MJ method. The size of the circles reflects the frequency of haplotypes, the transverse thin lines on the branches represent mutational events, and the thick black lines of varying widths represent insertions of 2, 6, or 9 bp.

0.172, respectively). Limited variability was found between geographically close populations of Primorskii krai and Northeastern China ($F_{ST}$ = 0.071), but the lowest values were between the pair Northeastern China–North China ($F_{ST}$ = 0.040). Such genetic structuring may reflect the dispersal routes of *E. senticosus* after the LGM in the direction from south to northeast. However, it can be assumed that some haplotypes could have survived the LGM in the refugi of the southern RFE, as indicated by the presence of unique (Esc2) and rare (Esc10, Esc11) haplotypes in the populations. Moreover, the total number of haplotypes in Primorskii krai (11) is higher than in Northern China (5), located further to the south. Climatic reconstructions in the work (Evstigneeva et al., 2025) confirm that during the LGM, forest–steppe landscapes were widespread in the southern part of Primorye and on the open shelf of Peter the Great Bay. The climatic parameters were slightly lower than modern values, and it is assumed that some species could have survived in protected mountain valleys. We have not found any literature data on whether conditions for the existence of *E. senticosus* on the southern part of Sakhalin Island during the LGM were suitable; it is possible that the species migrated to the Japanese islands, like representatives of thermophilic flora (Krestov et al., 2004), and then recolonized Sakhalin Island during periods of climatic warming. However, the presence of common haplotypes (Esc5, Esc6, Esc8), the dominance of the Esc7 haplotype on the island, and its rare presence in four mainland populations do not exclude any of these scenarios.

Thus, *E. senticosus* populations in the southern part of the Russian Federation are characterized by medium (Sakhalin Island) and high (Primorskii krai) levels of genetic diversity and low levels of genetic differentiation. All known haplotypes for *E. senticosus* in the Russian Federation and China form a single group; no phylogeographic structure was revealed. Haplotypes characteristic only of this region (Esc2, Esc4, Esc9, Esc11), one unique haplotype, and two rare haplotypes for the species were found on the territory of the Russian Federation. During the harvesting of raw materials, the plant is completely destroyed, which can lead to genetic drift from the population and the loss of genetic diversity. Recently, the popularity of naturopathy and the demand for natural adaptogens have increased, so the issue of preserving populations with high genetic diversity or transferring plants with rare and unique haplotypes to the territory of nature reserves has become relevant. The results of this study can be taken into account in the genetic monitoring of the state of populations of *E. senticosus*.

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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